IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of)
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Hawiger *et al.*) Group Art Unit: 1636
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Serial No. 09/516,310) Examiner: Loeb, B.
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Filed: March 1, 2000) Confirmation No. 3622
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For: A NOVEL METHOD FOR IMPORTING BIOLOGICALLY ACTIVE MOLECULES INTO CELLS)

DECLARATION OF JACK JACEK HAWIGER, M.D., PH.D., UNDER 37 C.F.R. § 1.132

I, Jack Jacek Hawiger, declare as follows:

1. I hold an M.D. from Copernicus School of Medicine, Jagiellonian University in Cracow, Poland, and a Ph.D. in Medical Microbiology from the National Institute of Hygiene in Warsaw, Poland. Since 1990, I have held the position of Oswald T. Avery Professor and Chair of the Department of Microbiology and Immunology at Vanderbilt University School of Medicine. Prior to this, I held the position of Professor of Medicine at Harvard Medical School. I am a Fellow of both the American Academy of Microbiology and the Infectious Diseases Society of America. I have authored and published over one hundred peer-reviewed scientific research articles and scientific review articles.

2. I am an inventor of the above-referenced patent application.

3. As described in the above-referenced application, the present invention relates to a method, pioneered in my laboratory, of importing molecules into cells via importation-competent signal peptides. My laboratory has been focused upon this area of research since 1992, and I have authored a number of peer-reviewed scientific research papers and review articles regarding this topic. In addition, I and my co-inventors have been awarded several patents relating to this invention.

4. U.S. Patent No. 6,495,518, of which I am an inventor, describes an experiment in which a functional peptide (denoted "SN50") was tested for its ability to enter cells and attenuate or prevent the inflammatory response *in vivo*, by interfering with the importation of NF-κB into the cells' nuclei. NF-κB is a pleiotropic activator that regulates expression of a number of cellular and viral genes, and, importantly, is a mediator of inflammation *in vivo*.

5. As described in the present patent application (page 38, line 25, through page 30, line 21), as well as in U.S. Patent No. 6,495,518, the SN50 peptide contains a hydrophobic sequence from the signal peptide of K-FGF, linked to a functional cargo peptide in the form of a nuclear localization signal (NLS) from NF-κB.

6. Administration of lipopolysaccharide (LPS) to mice is a well-known model for the study of septic shock, a systemic inflammatory condition. SN50 was first tested *in vitro* for its ability to block the importation of NF-κB in two types of cells known to be a target for LPS: macrophages and endothelial cells. We found that, for both of these cell types treated with the inflammatory inducer LPS, SN50 blocked the importation of LPS-induced NF-κB into the nuclei. Similar results were seen when another inflammatory inducer, i.e., TNF-α, was used. This result depended upon the presence of a functional NLS, because SM, a control peptide containing the signal sequence of SN50 but having a loss-of-function, mutated NLS, entered the cells but did not inhibit entry of NF-κB into the cells' nuclei.

7. The efficacy of SN50 peptide-directed inhibition of NF-κB and other stress-responsive activators in attenuating or preventing *in vivo* systemic inflammation and tissue injury was demonstrated by injecting mice intraperitoneally with D-galactosamine (20 mg) followed by LPS from *E. coli* serotype 0127:B8 (1 μg). (Mice treated with D-galactosamine are sensitive to low doses of LPS.) As shown in Fig. 2A from U.S. Patent No. 6,495,518 (Figs. 2A-2D from U.S. Patent No. 6,495,518 are attached herewith), all but one mouse showed symptoms of acute illness within 4 hours and died within six hours following injection of LPS. In contrast, as shown in Fig. 2C from U.S. Patent No. 6,495,518, mice treated with the SN50 peptide (5

injections up to 3.5 hours after LPS treatment) showed no symptoms of shock and survived the first 24 hours. By 48 hours, 50% of the mice survived and by 72 hours, 20% survival was observed. This *in vivo* protective effect is dependent upon a functional NLS domain, since it was abrogated when the SM peptide (with a mutated NLS domain) was used; specifically, all SM peptide-treated mice (5 injections up to 3.5 hours after LPS treatment) showed symptoms of acute illness and died with 5 hours (see Fig. 2B from U.S. Patent No. 6,495,518). Administration of SN50 peptide extended to 6 and 12 hours following LPS (7 intraperitoneal injections) resulted in 64% survival at 72 hours (Fig. 2D). The differences in survival are significant, with $P < 0.0001$ based on the log rank test.

8. As this model of systemic inflammation and tissue injury is characterized by fulminant liver injury, histopathologic analysis focused upon the liver. Sections obtained from mice receiving a lethal combination of D-galactosamine and LPS revealed diffuse hepatocellular injury with hallmarks of apoptosis (fragmented nuclei), engorgement of blood vessels filled with platelet thrombi, and extravasation of red blood cells. Identical patterns of massive apoptosis of hepatocytes accompanied by hemorrhagic liver necrosis were observed in mice treated with SM peptide. By contrast, tissue sections from the liver of SN50 peptide-treated mice that survived septic shock for 72 hours displayed almost normal liver architecture without any overt signs of apoptosis of hepatocytes or hemorrhagic necrosis. Therefore, administration of the SN50 peptide provides significant cytoprotection of the liver, a primary target organ in this model of septic shock and systemic inflammation and tissue injury.

9. In view of the above, I conclude that parenterally administered SN50 is systemically absorbed and crosses the plasma membrane of cells within a treated subject, thereby exerting its protective effect *in vivo*.

10. Based upon our experiments both *in vitro* and *in vivo*, as well as the experiments of other researchers, I further conclude that importation-competent signal peptides are an effective means for transporting biologically active molecules into cells in a subject.

11. I further declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or document or any patent issuing therefrom.

JACK JACEK HAWIGER, M.D., PH.D.

August 25, 2003
DATE